THIAMINE AND TETRAHYMENA

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Since the publication of our paper on the biosynthesis of thiamine by *Tetrahymena geleii* and *T. vorax* (Kidder and Dewey, 1942) we have expanded our investigations with regard to the basic medium employed. In the earlier work "vitamin-free casein" (Harris, highest chemical purity) was used exclusively as a base. This protein in a one per cent solution with added salts (modified Osterhout's solution) gave "very little growth" either alone or when any or all of the following vitamins were added: thiamine, riboflavin, pyridoxin, pantothenic acid, nicotinic acid, pimelic acid, i-inositol, uracil and p-aminobenzoic acid. Optimum growth resulted when extracts of plant leaves were added (Dewey, 1941; 1944). Even after the extracts were heated for one or more hours at 123° C. at pH 10–11.5 (to destroy thiamine) it was found that optimum growth again resulted without the addition of thiamine. It was further shown that thiamine had been synthesized by *T. geleii* by testing with *Glaucoma scintillans*, an organism dependent upon exogenous thiamine. A tentative conclusion was reached that there was, in the plant extracts, a factor which in some way made it possible for Tetrahymena to synthesize thiamine. This tentative factor was designated factor S.

It has recently been stated by Hall and Cosgrove (1944) that there was no evidence for the biosynthesis of thiamine by Tetrahymena on the basis that "vitamin-free" casein contains appreciable amounts of thiamine. They were able to obtain transplantable growth, using their strain of *T. geleii* (*Glaucoma piriformis*), in unsupplemented one per cent salted casein but not in heat- and alkali-treated salted casein unless thiamine was added. They believe that our low growth was due to a lack of minerals in the media used. Their criticism regarding the mineral factors is justified, as it was not clearly stated in our paper that salts (modified Osterhout's solution) were added. Inorganic salts were mentioned only in "substances used in the preparation of media."

The following experiments, modified to insure thiamine-free media, confirm our earlier results and again show that *Tetrahymena geleii* (strain W) is entirely independent of an exogenous source of thiamine for indefinitely transplantable growth. Quantitative results are being presented for the first time in this connection.

Material and Methods

Pure culture (bacteria-free) *Tetrahymena geleii* (strain W) was used exclusively in the present study. This is the strain which was used by us on previous occasions (Kidder, 1941a; Dewey, 1941; 1944; Kidder and Dewey, 1942). All experiments were carried out in chemically clean Pyrex tubes or flasks and aseptic technique was employed throughout. All media were prepared with water distilled twice over permanganate in an all-Pyrex still. The following substances were used:

casein (Harris, highest chemical purity); gelatin (Harris, selected grade, refined, vitamin-free); alfalfa leaf meal (Denver Alfalfa Milling and Products Co.); thiamine hydrochloride (Hoffman-LaRoche); riboflavin and l-tryptophane (Merck and Co.); inorganic salts (Baker and Adamson). All media were sterilized by autoclaving.

Base media

1. Heated casein—A two per cent solution (treated as described by Dewey, 1944) of casein was autoclaved for one hour at pH 10. After cooling and neutralizing the concentration was adjusted to one per cent or to 0.5 per cent. This medium is always quite turbid and a precipitate settles out upon standing, so the available casein is considerably reduced from the figures given.

2. Filtered heated casein—Heated casein was allowed to cool, the pH adjusted to 6.8, and the precipitate removed by filtration through a Buchner filter with the aid of Celite (Johns-Manville). The filtrate was then diluted to what would be 0.5 per cent (calculated on the original casein). This gave a light straw colored clear solution which again precipitated slightly upon final sterilization.

3. Casein hydrolysate—Two per cent casein was refluxed 22 hours in a 24 per cent solution of H_2SO_4. The sulphate was removed with $Ba(OH)_2$. The resulting hydrolysate was biuret negative. This hydrolysate was used in a 0.5 per cent concentration (calculated from the original amount of casein used).

4. Gelatin—This was used in a two per cent solution.

5. Heated gelatin—Four per cent gelatin was autoclaved one hour at pH 10. After cooling the pH was adjusted to 6.8 and the solution was diluted to a concentration of two per cent.

6. Gelatin hydrolysate—Four per cent gelatin was refluxed for five hours in a 24 per cent solution of H_2SO_4. The sulphate was removed by $Ba(OH)_2$. This hydrolysate was biuret negative and was used in one per cent concentration (calculated from the original amount of gelatin used).

Alfalfa extract was prepared as described previously (Kidder and Dewey, 1942). After heat and alkali treatment at pH 10 it was adjusted to pH 6.8 and added in a dilution of 1 : 10 final concentration. This dethiaminized extract is designated A.

Thiamine hydrochloride was added where indicated in the concentration of one microgram per ml. of medium.

To all of the media used in the following experiments were added just before sterilization the following inorganic salts (Hall and Cosgrove, 1944): 0.02 per cent $MgSO_4 \cdot 7H_2O$; 0.02 per cent K_2HPO_4; 0.01 per cent $CaCl_2 \cdot 2H_2O$; 0.00025 per cent $FeCl_2 \cdot 6H_2O$; 0.00001 per cent $MnCl_2 \cdot 4H_2O$; 0.00001 per cent $ZnCl_2$. To all media was also added 0.1 microgram per ml. of riboflavin. Tryptophane was added to the hydrolysed casein (to compensate for loss in hydrolysis) and to all gelatin and gelatin hydrolysates to a concentration of 0.0025 per cent. Experiments with amino acid mixtures now being conducted show that tryptophane is essential to the growth of Tetrahymena. All media were used at pH 6.8–6.9.

A number of preliminary experiments were carried out with each medium in tubes in serial transplants. Each tube contained five ml. of medium. All tube series were inoculated with a bacteriological loop delivering approximately 0.008 ml. Tube series were grown through at least three transplants before any conclusions

were drawn, this to eliminate the possibility of carry over of medium from the stock cultures. Tube cultures were incubated at room temperature and transplants were made every 48 hours, except where very slow growth occurred in the early transplants, where longer times were allowed.

The quantitative studies were made using the culture flasks described earlier (Kidder, 1941b). These flasks contained 100 ml. of media. Inoculations were made from third transplant tubes of like media so that the flask cultures represent fourth transplant series. Sterile serological pipettes were used for the inoculations and from 0.1 ml. to 0.5 ml. was added, depending upon the density of the population in the tube from which the inoculation was made. After the first few experiments inoculations were made from cultures within the exponential growth phase and the inoculations were calculated to give an initial count of as near 100 cells per ml. as possible. Flask cultures were incubated at 24.5° C. All flask experiments were repeated at least once.

Our method of counting cells from culture has been described elsewhere (Kidder, 1941b), but it should be noted here that this method gives only viable counts, hence our population counts tend to be lower in the stationary phase and phase of decline than where methods involving the counting of killed cells is employed. These differences are well illustrated in the work of Johnson and Baker (1943).

Generation time (g) was calculated by the use of the formula

$$g = \frac{t \log 2}{\log b - \log a}$$

where t = the time in hours during which the population has been increasing exponentially, a = the number of cells per unit volume at the beginning, and b = the number of cells at the end of time, t.

Experimental

Population Studies

Casein and casein hydrolysate—When a solution of casein is adjusted to pH 10 and autoclaved for one hour to render it thiamine-free, a number of changes take place which make it very inferior to unheated casein as a basic medium for Tetrahymena. Hall and Cosgrove (1944) state that factors in addition to thiamine must have been destroyed, because even upon the addition of thiamine poor growth resulted. With our strain of *T. geleii* heat treated casein plus thiamine (also salts and riboflavin, as mentioned above) inhibited growth even in the first transplant, and second transplants were almost invariably negative. In no case was growth obtained in the third transplant. However, if the insoluble precipitate resulting from such treatment is filtered off and the concentration (originally one per cent before filtration) is reduced by one-half then low but transplantable growth results. The addition of thiamine has no significant effect upon the generation time, length of the logarithmic phase, maximum yield or survival up to the limit of our experiment (Table I; Fig. 1). This indicates that the heat treatment has produced toxic substances which, when reduced in concentration do not inhibit growth entirely. It also shows that Tetrahymena can reproduce without an exogenous source of thiamine.

TABLE I

Medium	Generation time in hours	Population per ml. at end of log. phase	Maximum yield cells/ml.	Population per ml. at 11 days
Heated casein 0.5 per cent + A	5.02	18,000	160,000	61,000
Heated casein 0.5 per cent + A + B_1	4.80	17,500	182,000	82,000
Filtered heated casein	8.35	1,600	5,500	2,000
Filtered heated casein + B_1	8.96	1,800	8,000	3,200
Filtered heated casein +A	4.43	42,000	100,000	57,000
Filtered heated casein + A + B_1	4.27	38,000	110,000	86,000

A = heat and alkali treated alfalfa extract; B_1 = thiamine 1 microgram/ml. All media contains salts and riboflavin (0.1 micrograms/ml.).

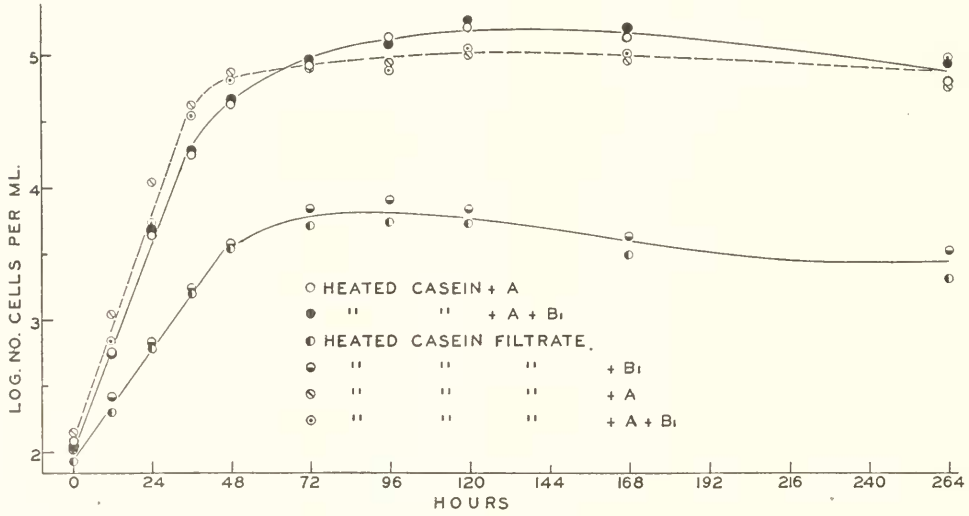

FIGURE 1

When dethiaminized alfalfa extract is added to the heated casein or to the filtered heated casein the response is striking. Rapid growth now occurs in the heated casein while in the filtered heated casein the generation time is reduced by nearly one-half and the population at the end of the logarithmic growth phase is increased from around 1,600 to over 40,000 per ml. The maximum yield is increased from about 5,000 to approximately 100,000 per ml. and a much higher population is maintained for at least 11 days (over 50,000 as compared to 2,000 per ml.) (Table

I; Fig. 1). This would seem to indicate that, in addition to supplying stimulatory factors (Dewey, 1944) and the synthesizing factor, the alfalfa extract counteracts the toxic effects of the heat treatment on the casein. There is indication in the shape of the growth curve that the greater toxicity of the heated casein has not been as successfully counteracted as that of the filtered heated casein. The generation time is approximately 0.5 hour longer in the former and the population begins to fall off sooner. The maximum yield, however, is higher (160,000 as compared to 100,000 per ml.) in the unfiltered casein. This last may be due to the higher concentration of available protein.

TABLE II

Medium	Generation time in hours	Population per ml. at end of log. phase	Maximum yield cells/ml.	Population per ml. at 11 days
Casein hydrolysate 0.5 per cent + A	4.27	50,000	122,000	49,000
Casein hydrolysate 0.5 per cent + A + B_1	4.36	31,000	191,000	94,000
Heated casein hydrol. 0.5 per cent + A	4.37	29,000	182,000	54,000
Heated casein hydrol. 0.5 per cent + A + B_1	4.46	32,000	171,000	73,000

A = heat and alkali treated alfalfa extract; B_1 = thiamine 1 microgram/ml. All media contains salts, riboflavin (0.1 microgram/ml.) and l-tryptophane (0.0025 per cent).

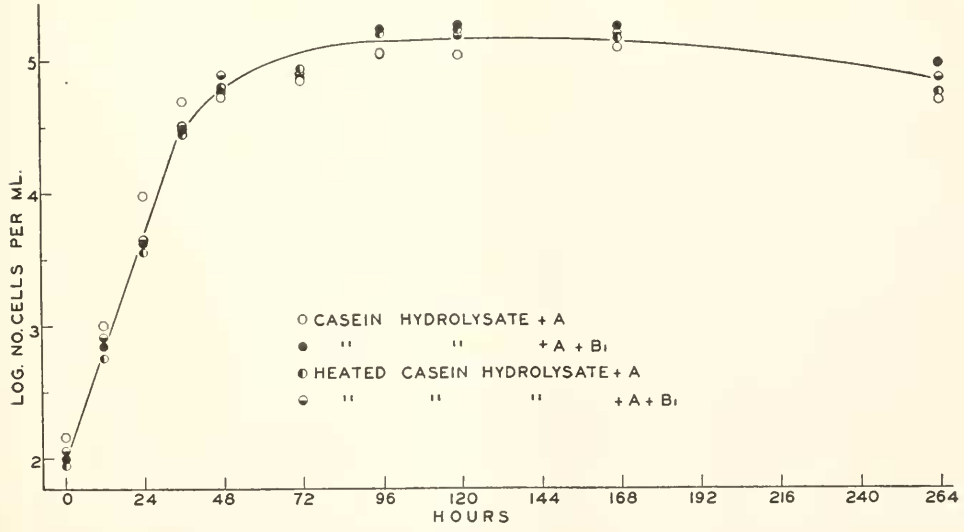

FIGURE 2

The addition of thiamine to either the heated casein or filtered heated casein plus dethiaminized alfalfa extract has very little effect. The cultures maintain a slightly higher level at 11 days duration but the shape of the growth curves are almost identical.

When a 0.5 per cent solution of a biuret negative casein hydrolysate plus 0.0025 per cent l-tryptophane was used as a basic medium it was found that growth was impossible beyond the first transplant, even when thiamine was added. Inasmuch as unheated casein and filtered heated casein give slow but indefinitely transplantable growth the acid hydrolysis must have destroyed some factor or factors, other than thiamine, necessary for growth. Excellent growth resulted, however, when de-

TABLE III

Medium	Generation time in hours	Population per ml. at end of log. phase	Maximum yield cells/ml.	Population per ml. at 11 days	Size of cells at 11 days (av. 20 measurements)
Gelatin 2 per cent	5.68	2,600	12,000	2,100	$22\mu \times 16.5\mu$
Gelatin 2 per cent + B_1	5.59	2,400	67,000	31,000	$91.5\mu \times 34\mu$
Gelatin 2 per cent + A	3.21	18,000	140,500	47,000	$51\mu \times 24\mu$
Gelatin 2 per cent + A + B_1	3.22	17,200	161,000	52,000	$86.5\mu \times 22\mu$

A = heat and alkali treated alfalfa extract; B_1 = thiamine 1 microgram/ml. All media contains salts, riboflavin (0.1 microgram/ml.) and l-tryptophane (0.0025 per cent).

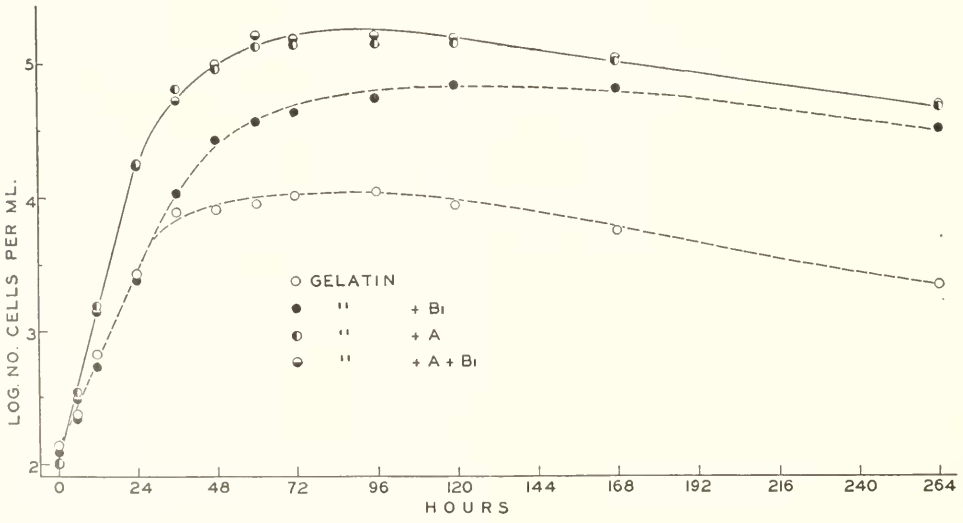

FIGURE 3

TABLE IV

Medium	Generation time in hours	Population per ml. at end of log. phase	Maximum yield cells/ml.	Population per ml. at 11 days	Size of cells at 11 days (av. 20 measurements)
Heated gelatin 2 per cent	5.31	2,750	11,700	200	$20\mu \times 16\mu$
Heated gelatin 2 per cent + B_1	5.72	2,000	68,500	44,000	$85\mu \times 30\mu$
Heated gelatin 2 per cent + A	3.08	19,500	82,000	40,000	$47\mu \times 20\mu$
Heated gelatin 2 per cent + A + B_1	3.42	17,500	96,000	72,000	$89\mu \times 36.5\mu$

A = heat and alkali treated alfalfa extract; B_1 = thiamine 1 microgram/ml. All media contains salts, riboflavin (0.1 microgram/ml.) and l-tryptophane (0.0025 per cent).

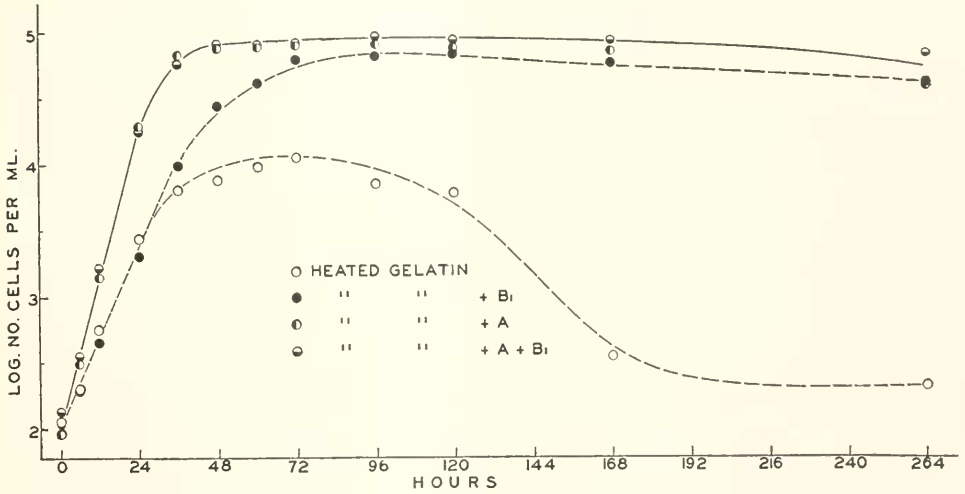

FIGURE 4

thiaminized alfalfa extract was added, and again the addition of thiamine had no effect except upon survival (Table II; Fig. 2).

Gelatin and gelatin hydrolysate—Gelatin Harris is a purified product, according to its manufacturers. It was found, however, that fair growth could be maintained in a one per cent or a two per cent solution provided tryptophane was added. The generation time was about 5.5 hours, the population at the end of logarithmic growth about 2,500, the maximum yield about 12,000 and at the end of 11 days the population was approximately 2,000 per ml. The addition of thiamine had no effect on the generation time or the length of the logarithmic phase but the maximum yield was increased to 67,000 per ml. while at the end of 11 days over 30,000 ciliates per ml. were present (Table III; Fig. 3). This might indicate that enough thiamine

was present for limited growth in the unsupplemented gelatin and that added thiamine was necessary for the increased maximum yield and higher survival.

When dethiaminized alfalfa extract is added to gelatin plus tryptophane the generation time is reduced to a little over three hours, the population at the end of logarithmic growth is increased to about 18,000, the maximum yield is increased to over 140,000 and the population at the end of 11 days is increased to 47,000 per ml. (Table III; Fig. 3). This shows the stimulatory effect of the alfalfa factors. The

TABLE V

Medium	Generation time in hours	Population per ml. at end of log. phase	Maximum yield cells/ml.	Population per ml. at 11 days	Size of cells at 11 days (av. 20 measurements)
Gelatin hydrolysate 1 per cent + A	4.22	57,500	195,000	8,500	$50.5\mu \times 27\mu$
Gelatin hydrolysate 1 per cent + A + B_1	4.26	51,500	208,000	10,000	$99\mu \times 41\mu$
Heated gelatin hydrolysate 1 per cent + A	4.59	54,500	160,000	11,000	$42.5\mu \times 19\mu$
Heated gelatin hydrolysate 1 per cent + A + B_1	4.62	31,000	321,000	9,200	$78\mu \times 35\mu$

A = heat and alkali treated alfalfa extract; B_1 = thiamine 1 microgram/ml. All media contains salts, riboflavin (0.1 microgram/ml.) and l-tryptophane (0.0025 per cent).

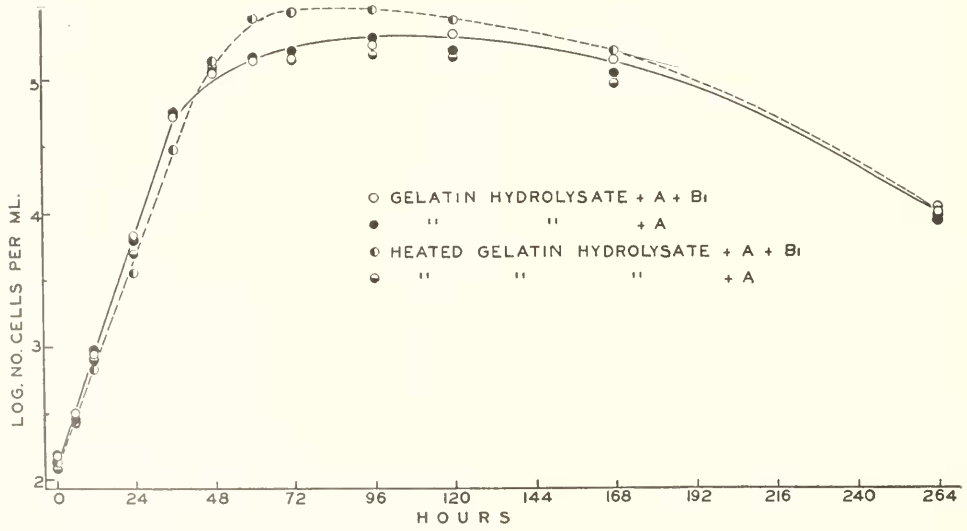

FIGURE 5

addition of thiamine to gelatin-tryptophane plus dethiaminized alfalfa extract had no significant effect.

When dethiaminized gelatin plus tryptophane is used the only apparent difference from unheated gelatin is in the survival. The ciliate population in heated gelatin begins to decrease more rapidly until at the end of 11 days only about 200 per ml. remain (Table IV; Fig. 4). In eighth transplant tube cultures viable ciliates were found after 30 days. There can be no question that *Tetrahymena geleii* (strain W) does not require an exogenous source of thiamine for limited growth in this medium. Tube cultures were carried through six transplants using glass wool instead of the usual washed and bleached cotton for stoppers, in order to be sure that no trace of thiamine could enter the medium from the few cotton fibers which sometimes drop from the stoppers. All factors required for thiamine synthesis by the ciliates must be present in limited amounts in gelatin and must withstand the rigorous heat and alkali treatment. The addition of thiamine or alfalfa extract or both to heated gelatin produced about the same results as when added to unheated gelatin (Table IV; Fig. 4).

Acid hydrolysis of gelatin destroys factors necessary for the growth of *Tetrahymena geleii* (strain W) for in no case was growth maintained beyond the initial transplants without the alfalfa factors. This is comparable with the casein hydrolysate. The addition of thiamine had no effect.

Excellent growth resulted when dethiaminized alfalfa extract was added to gelatin hydrolysate plus tryptophane. Best growth was obtained in a one per cent solution of the hydrolysate although two per cent gave only slightly lower growth. The addition of thiamine to the hydrolysate plus alfalfa extract had no effect (Table V; Fig. 5).

Heating the gelatin hydrolysate at 123° C. for one hour at pH 10 caused slight changes of doubtful significance. Again excellent growth occurred upon the addition of dethiaminized alfalfa extract and again the addition of thiamine had no effect on population growth (Table V; Fig. 5). This again demonstrates that these ciliates do not require exogenous thiamine for continued growth. In tube cultures the tenth transplant (in heated gelatin hydrolysate plus tryptophane and dethiaminized alfalfa extract) behaves like the first transplant, and with the technique employed there can remain no effective carry over from the stock solution.

Ciliate Size Relations

The above discussion has been concerned only with population growth. The individual cells vary in size and shape to a great extent depending upon the type of medium employed. This variation is never apparent, however, during active multiplication, but only during the stationary phase and the period of population decline. Detailed observations and measurements were made on cultures based on gelatin and gelatin hydrolysate. The differences noted were due to the presence or absence of the alfalfa extract or of thiamine and not to the nitrogen source.

When the ciliates have grown for from 8 to 11 days in a medium lacking both the alfalfa factors and thiamine (outside supply) they become very small (Tables III and IV) and evenly piriform. The protoplasm appears somewhat dense and the motion of the ciliates is very reduced. The small size can be detected with the naked eye in tube cultures. These small ciliates are perfectly viable, however, and normal cultures result upon transplantation into fresh medium.

When thiamine is added to the medium the ciliates increase in size during the stationary period until at the end of 11 days (the time when measurements were taken) they may be as much as 100 μ in length (Tables III and IV). Most of them are flattened and irregular in shape, are fairly active and quite transparent.

When the alfalfa extract is added to the base medium the ciliates in the stationary period and period of decline are about midway in size between those in media with neither alfalfa nor thiamine and those in the media with added thiamine (Table III, IV and V). These ciliates are also flattened and transparent and are actively motile.

When both the alfalfa factors and thiamine are added together large ciliates result. These are about the size and appearance of those in cultures where only thiamine is added (Tables III, IV and V). They are more actively motile, however.

The above observations are preliminary and limited in nature but they indicate differences due to accessory factors and warrant more detailed study.

Discussion

From the results of our experiments with media based on casein and gelatin we can offer the following statements regarding thiamine and *Tetrahymena geleii* (strain W). In a dethiaminized medium of gelatin plus tryptophane or in dethiaminized casein (filtered) plus inorganic salts and riboflavin this ciliate can be serially transplanted apparently indefinitely. It appears to be able to synthesize thiamine enough for moderate to low population growth. This synthetic activity seems to be in direct ratio to the amount of some substance (factor S of Kidder and Dewey, 1942) present in small amounts in the heat-treated casein and gelatin. When thiamine is added to the gelatin medium no effect is shown during the logarithmic phase. But as the factors catalyzing the synthesis of thiamine are being depleted (end of logarithmic phase), reproduction falls off sharply in the gelatin medium alone but continues further for some two and a half divisions when outside thiamine is provided. The presence of toxic products of the heat and alkali treatment on casein makes this medium so unsuitable for the ciliates that growth is limited even with added thiamine.

The raising of the reproductive rate by the addition of "factors I and II" (Dewey, 1944) contained in the alfalfa extract counteracts the toxicity of the heat and alkali treated casein. Also large amounts of "factor S" are made available for the synthesis of thiamine over a longer period of time. Indeed this synthesis is enough to meet the requirements for rapid growth and the addition of an outside supply of thiamine has no effect, until the period of population decline.

It seems evident that the denial by Hall and Cosgrove (1944) of our previous conclusions (that *T. geleii* can synthesize thiamine) can now be dismissed. There can be no question of the thiamine-free nature of our medium. A point of some interest, however, is the fact that they obtained transplantable growth with their strain of Tetrahymena (*Glaucoma piriformis*) in "1 per cent dethiaminized casein" plus salts and thiamine but not when thiamine was omitted. Our strain (W) failed to grow in this strength either with or without thiamine. Strain differences may account for this apparent discrepancy, as we found that *T. geleii* (Hetherington strain), the one used in previous studies in this laboratory (Kidder, 1941b; Kidder and Stuart, 1939; Kidder, Lilly and Claff, 1940), is more resistant to the

toxic substance produced by the heat and alkali treatment on casein and grew in serial transplants very slowly and in low concentration in 1 per cent heated casein plus thiamine, riboflavin and salts. Another factor which must be taken into consideration is the fact that Hall and Cosgrove discarded the precipitate after decanting the supernatant fluid from heat and alkali treated 1 per cent casein. This would make their medium similar to our filtered heated casein. In view of the activity of the ciliates in heated gelatin where toxicity is less pronounced we believe the principal effect of thiamine in this case as well as in the case recorded by Hall and Cosgrove was to detoxify the medium. We have similar data with various amino acids in other types of experiments now being carried on. Had Hall and Cosgrove repeated our experiments (1942) by using dethiaminized alfalfa extracts they would not, in all probability, have stated that "the results obtained with Glaucoma piriformis afford no basis for concluding that this ciliate synthesizes thiamin. . . ."

We are in agreement with Hall and Cosgrove (1944) regarding the mineral requirements of *Tetrahymena* and accordingly the inorganic salts were always added. We do not know the specific effect of riboflavin on strain W but knowing that this vitamin is rapidly destroyed in alkaline solution by light it was thought best to add sufficient amounts to insure against its being a limiting factor.

In our previous report (Kidder and Dewey, 1942) we stated that "factor S" had not been detected in material of animal origin. It was pointed out, however, that the production of toxic substances by the heat and alkali treatment might mask the presence of "factor S." We are now of the opinion that small amounts of "factor S" are present in Gelatin Harris and Casein Harris and possibly associated with other animal proteins.

In this study we have assumed the synthesis of thiamine by Tetrahymena on the basis of transplantable growth in completely dethiaminized media. This assumption might be questioned on the basis that there is a possibility that Tetrahymena does not use thiamine in its metabolic activities. As far as we know it would then be unique among plants and animals. It must also be remembered that evidence was presented previously (Kidder and Dewey, 1942) that thiamine was actually synthesized and could be detected by the use of *Glaucoma scintillans,* a thiamine-requiring ciliate. Also in view of the real effects adequate amounts of thiamine have on the population levels and on survival (see Johnson and Baker, 1943) and on size, the inclusion of thiamine in the metabolism of this ciliate is almost certain.

One point of importance which should be discussed here is our success in obtaining growth in completely hydrolysed proteins. Lwoff (1932) found that *"Glaucoma piriformis"* would not grow in abiuretic media. He concluded tentatively that these ciliates require peptides or more complex molecules in addition to growth factors. He did not use anything which would correspond to our alfalfa extract. It seems now that hydrolysis does destroy growth factors which can be re-introduced by the addition of heat-treated alfalfa. The whole problem of the nitrogen requirements is being investigated more thoroughly in this laboratory and will be reported elsewhere, but it can be stated from experiments with amino acids that the theory of the "peptide requirement" of Tetrahymena is no longer tenable. Kline (1943) came to the same conclusion.

On the basis of our experiments we can say that thiamine is no longer to be regarded as a "growth factor", as defined by Lwoff (1936–37), for *Tetrahymena geleii*. In the presence of adequate amounts of a certain substance or substances of unknown chemical nature, which we have called "factor S," thiamine is synthesized in sufficient quantity to insure rapid and heavy growth. We offer the suggestion that the great size differences noted in declining cultures with and without added thiamine is related to an ultimate depletion of "factor S," and hence thiamine, in the completely dethiaminized media.

It is apparent from a comparison of generation times and population densities that gelatin based media are as good or better than those based on casein. This is only true, however, when gelatin is supplemented with tryptophane. In view of the ease with which gelatin can be handled it is to be preferred to casein.

Summary

1. *Tetrahymena geleii* will grow in serial transplants in completely dethiaminized filtered and diluted casein solution plus salts and riboflavin. Added thiamine has no effect upon the population.

2. Growth rate, population at the end of exponential growth and maximum yield are greatly increased when dethiaminized alfalfa extract is added.

3. No growth occurs in completely hydrolysed casein even with added thiamine.

4. Good growth occurs in casein hydrolysate plus dethiaminized alfalfa extract. Heat and alkali treatment of the hydrolysate or the addition of thiamine have no significant effect.

5. Whole gelatin and dethiaminized whole gelatin support transplantable growth. The addition of thiamine has no effect during logarithmic growth but causes an increase in maximum yield.

6. The addition of dethiaminized alfalfa extract to gelatin and to dethiaminized gelatin increases significantly the reproductive rate, population at the end of logarithmic growth and the maximum yield.

7. Gelatin hydrolysate and dethiaminized gelatin hydrolysate support growth only when alfalfa extract is added. The addition of thiamine has no significant effect.

8. Ciliates growing in gelatin or heated gelatin alone become extremely small and sluggish after about the eighth day. They remain viable up to 30 days, however. The addition of thiamine causes the ciliates to become very large in old cultures. The addition of alfalfa extract produces ciliates intermediate in size.

9. The conclusion is reached that casein and gelatin possesses small amounts of "factor S" which makes it possible for *Tetrahymena geleii* to synthesize thiamine. "Factor S" from alfalfa extract, together with "factors I and II" (Dewey, 1944) added to casein or gelatin, produce rapid and heavy growth. Thiamine is not a "growth factor" for *T. geleii* (strain W).

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